Claims

1. Method for selecting mutated O_R or O_L operator DNA sequences from lambdoid phages which have a different thermostability compared to the wild-type sequence with regard to binding a repressor,
wherein
(a) a DNA cassette is prepared which contains a selection gene under the operative control of an expression control sequence comprising at least one O_R or O_L operator sequence from a lambdoid phage and a promoter,
(b) the operator DNA sequence is subjected to a mutagenesis and
(c) the mutated operator DNA sequences are analysed.

2. Method as claimed in claim 1,
wherein
the lambdoid phages are selected from the group comprising the phage lambda, phage 21, phage 22, phage 82, phage 424, phage 434, phage D326, phage DLP12, phage gamma, phage HK022, phage P4, phage Phi80, phage Phi81, coliphage 186 and recombinant variants thereof.

3. Method as claimed in claim 2,
wherein
the phage lambda or recombinant variants thereof are used.

4. Method as claimed in claim 3,
wherein
an operator DNA sequence from the operator regions O_R or/and O_L of the phage lambda is used.

5. Method as claimed in one of the claims 1 - 4,
wherein
the E-lysis gene from the phage PhiX174 is used as the selection gene.

6. Method as claimed in one of the claims 1 - 5,
wherein
the operator DNA sequence is subjected to a site-specific mutagenesis by oligonucleotides or a selection is carried out in a mutator bacterial strain.

7. Method as claimed in one of the claims 1 - 6,
wherein
the mutated operator DNA sequences are analysed by determining their ability to bind to a temperature-sensitive cI repressor.

8. Method as claimed in claim 7,
wherein
the temperature-sensitive lambda repressor cI857 is used.

9. Mutated O_R or O_L operator sequences from lambdoid phages which have a different thermostability compared to the wild-type sequence with regard to binding of a repressor and are obtainable by a method as claimed in one of the claims 1 - 8.

10. Mutated O_R or O_L operator sequences from lambdoid phages which have an increased thermostability compared to the wild-type sequence with regard to binding of a temperature-sensitive repressor and are obtainable by a method as claimed in one of the claims 1 - 8.

11. Mutated O_R or O_L operator sequence as claimed in claim 10,
wherein
it has an approximately 3 - 10°C increased thermostability.

12. Mutated O_R or O_L operator sequence as claimed in claim 10,
wherein
it has an approximately 7 - 9°C increased thermostability.

13. Mutated lambda O_R or O_L operator sequence as claimed in one of the claims 9 - 12, which is a variant of the sequences shown in SEQ ID NO.1 or SEQ ID NO.3.

14. Mutated lambda O_R operator sequence comprising the sequence shown in SEQ ID NO.2.

15. Use of a mutated O_R or O_L operator sequence as claimed in one of the claims 9 - 14 for the temperature-regulated expression of genes in bacterial cells.

16. Use of a combination of (a) a wild-type O_R or O_L operator region and at least one operator region which contains a mutated O_R or O_L operator sequence as claimed in one of the claims 9 - 14 or (b) several operator regions which contain mutated O_R or O_L operator sequences as claimed in one of the claims 9 - 14 with different thermostabilities for the temperature-regulated sequential expression of genes.

17. Use as claimed in claim 15 or 16,
wherein
the bacterial cells contain a gene for a cI repressor from lambdoid phages for the regulation of gene expression.

18. Use as claimed in claim 17,
wherein
the bacterial cells contain the gene for the lambda cI857 repressor.

19. Nucleic acid comprising a bacterial expression control sequence which contains a mutated O_R or O_L operator sequence as claimed in one of the claims 9 - 14 in operative linkage with a protein-coding sequence.

20. Nucleic acid as claimed in claim 19,
wherein
the protein-coding sequence is a suicide gene.

21. Nucleic acid as claimed in claim 20,
wherein
the expression control sequence contains a lambda P_L or P_R promoter.

22. Vector,
wherein
it contains at least one copy of a nucleic acid as claimed in one of the claims 19 - 21.

23. Vector as claimed in claim 22,
wherein
it is a bacterial chromosomal vector.

24. Vector as claimed in claim 22,
wherein
it is a bacterial extrachromosomal plasmid.

25. Bacterial cell,
wherein
it is transformed with a nucleic acid as claimed in one of the claims 19 - 21 or with a vector as claimed in one of the claims 22 - 24.

26. Bacterial cell as claimed in claim 25,
wherein
it contains the nucleic acid or the vector integrated into its chromosome.

27. Bacterial cell as claimed in claim 25 or 26,
wherein
it additionally contains a gene for a cI repressor from lambdoid phages.

28. Bacterial cell as claimed in claim 27,
wherein
it contains the gene for the lambda cI857 repressor.

29. Vaccine composition,
wherein
it contains a live bacterial cell as claimed in one of the claims 26 - 28 as an active ingredient optionally with pharmaceutically acceptable auxiliary substances, additives and carrier substances.

30. Vaccine composition,
wherein
it contains a bacterial ghost as the active ingredient optionally with pharmaceutically acceptable auxiliary substances, additives and carrier substances in which the bacterial ghost can be obtained by culturing a bacterial cell as claimed in one of the claims 25 - 28 at temperatures of 35 - 39°C and subsequently lysing the bacterial cell by increasing the temperature.

31. Nucleic acid comprising (a) a first bacterial expression control sequence which contains an O_R or O_L operator sequence from a lambdoid phage and to which a first cI repressor from lambdoid phages can bind, in operative linkage with a sequence coding for a second repressor wherein the second repressor cannot bind to the first bacterial expression sequence and (b) a second bacterial expression control sequence to which the second repressor can bind in operative linkage with a suicide gene.

32. Bacterial cell,
wherein
it contains at least one copy of a nucleic acid as claimed in claim 31.

33. Bacterial cell as claimed in claim 32,
wherein
it additionally contains a gene for the first repressor.

34. Bacterial cell as claimed in claim 32 or 33,
wherein
it contains the first bacterial expression control sequence of a mutated operator sequence as claimed in one of the claims 9 - 14.

35. Bacterial cell as claimed in one of the claims 32 - 34 additionally comprising (c) a third bacterial expression control sequence which contains a mutated operator sequence as claimed in one of the claims 9 - 14 in operative linkage with a suicide gene.

36. Vaccine composition,
wherein
it contains a live bacterial cell as claimed in one of the claims 32 - 35 as the active ingredient optionally together with pharmaceutically acceptable auxiliary substances, additives and carrier substances.

37. Use of vaccine compositions as claimed in claim 29 or 36 as heat-sensitive or/and cold-sensitive safe live vaccines.

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